In Vitro Androgen Receptor Transcriptional Activation Assay (ARTA)

Need

Endocrine disruption is of regulatory importance globally and is a primary consideration in determining the safety and/or efficacy of a dermatological drugs, cosmetics, chemicals, and agrochemicals. The androgenic pathway involves interaction of androgens with androgen receptors (ARs) that subsequently affect transcription of androgen-controlled gene expression. Compound or chemical perturbation of normal androgenic transcriptional activation pathways may have adverse effects on normal development, reproductive health, and/or the integrity of the reproductive system.

For new and existing drugs, cosmetics, chemicals, and agrochemicals, the cost and time necessary to assess disruption of the androgenic transcriptional activation pathways *in vivo* is prohibitory to efficient product development and safety assessment.

Solution

LifeNet Health LifeSciences offers the ARTA assay for the assessment of potential androgenic activation of test compounds or chemicals. In the ARTA assay, the MDA-kb2 cell line is used. This cell line has a stably inserted construct; a firefly luciferase reporter construct containing Androgen-Responsive Element (ARE) and expresses high levels of functional AR protein. Upon ligand binding, the ARs translocate to the nucleus where they interact with the ARE on the on the firefly luciferase construct, resulting in expression of the firefly luciferase enzyme. This enzyme transforms the luciferin substrate to a bioluminescent product that can be quantitatively measured with a plate reader. Using the basic procedures outlined below, the luminescent signal (i.e., AR transcriptional activation) and cell viability are determined immediately following exposure to test chemicals or compounds. This assay is performed for both agonism (activating AR specific gene expression) and antagonism (inhibiting AR specific gene expression). At least two runs will be conducted for both the agonism and the antagonism assays to determine if the test article(s) are positive or negative for AR transactivation (Tables 1 and 2).

Accurate & reliable data

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Unsurpassed expertise

Collaborative approach

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Standard Protocol

ASSAY PARAMETERS	PROTOCOL
Model	MDA-kb2 cell line
Replicates	3
Solvent of Choice	DMSO (preferred), sterile water, culture medium, or ethanol
Test Article Formulation	1 mM, 100 µM, 10 µM, 1 µM, 100 nM, 10 nM, 1 nM, 100 pM, and 10 pM (depending on solubility and cytotoxicity pre-testing)
Solvent Controls	DMSO (or sterile water)
Agonism Positive Control	10 nM 5α-Dihydrotestosterone
Agonism Reference Controls	5α-Dihydrotestosterone, Mestanolone, and Di(2-ethylhexyl)phthalate
Antagonism Positive Control	1 µM Hydroxyflutamide
Antagonism Reference Controls	Hydroxyflutamide, Bisphenol A, and Di(2-ethylhexyl)phthalate
Exposure Time	24 ± 2 hours
Cell Viability Assessment	MTT assay
ARTA Induction/Inhibition	Luminescence assay
Time to Complete	4-6 weeks from test article receipt
Regulatory	Non-GLP or GLP
Deliverables	Full Report, Agonism (PC_{10}, PC_{50} and RPC_{max}), Antagonism (IC_{30} and IC_{50})

Key References

Wilson et al. (2002) A Novel Cell Line, MDA-kb2, That Stably Expresses an Androgen- and Glucocorticoid-Responsive Reporter for the Detection of Hormone Receptor Agonists and Antagonists. Tox Sci 66(1) 69-81.

Table 1. AR agonist

Positive	If the RPC_{Max} is obtained that is equal to or exceeds 10% of the response of the positive control in at least two of two or two of three runs.
Negative	If the RPC_{Max} fails to achieve at least 10% of the response of the positive control in two of two or two of three runs.

Table 2. AR antagonist

Positive	If the IC_{30} is calculated in at least two of two or two of three runs.
Negative	If the IC_{30} fails to calculate in two of two or two of three runs..

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